



A Research Article

**Clinical, Hormonal and Genetics Features in patients with Androgen
Insensitivity Syndrome in Cytogenetic Laboratories in Semarang**

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Clinical, Hormonal and Genetics Features of Patients with Androgen Insensitivity Syndrome in Semarang

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Abstract

Androgen insensitivity syndrome (AIS) is an X-linked disorder caused by impaired Androgen Receptor (AR) which is encoded in Xq 11-12. In this condition, although the androgen is produced sufficiently, but the peripheral masculinizing effect is blocked, therefore sexual ambiguity occurred. Lack of information and knowledge of both medical personnel and community made AIS frequently misdiagnose and mismanagement.

This was a descriptive retrospective and prospective study following the clinical, hormonal, cytogenetic, and molecular study from subjects with suspected AIS from the molecular and cytogenetic laboratories in Semarang during April 2004 to April 2005. Medical record was obtained from Molecular and Cytogenetic Unit in Medical Faculty of Diponegoro University and Cytogenetic Laboratory of Telogorejo Hospital.

Quigley stage measurement in 46 PAIS cases were found in stage 3 for 18 (39,1%); in stage 2 for 16 cases (34,8%); in stage 4 for 6 cases (13%); in stage 6 for 3 cases (6,5%); in stage 1 for 2 cases (4,3%) and in stage 5 for 1 case (2,2%). Twelve individuals were raised as female whose 2 of them had been operated for gender adjustment to male. There were two large families with familial PAIS suggested an X-linked inheritance. Eight subjects (16,33%) showed the characteristic of PAIS hormone. Four out of nine subjects who had been examined using molecular analysis showed missense mutation R840H and I603N.

The diagnosis of PAIS need a complex assessment, not only based on physical examination but also should be considered using hormonal, cytogenetic and molecular analysis. The majority of patients came from low socioeconomic background, expensive cost and the availability of hormonal and molecular analysis in Indonesia is the main constraint in establishing the diagnosis.

Keywords : Androgen Insensitivity Syndrome, clinical features, hormones, AR-gene mutations.

Gambaran Klinis, Hormon dan Genetik pada Pasien dengan *Androgen Insensitivity Syndrome* di Semarang

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Abstrak

Sindrom androgen insensitif (AIS) merupakan kelainan X-link disebabkan karena terganggunya androgen reseptor (AR) yang dikode di Xq 11-12. Pada kondisi ini, meskipun produksi androgen mencukupi namun efek maskulinisasi perifer terhambat, dan berakibat pada ambiguitas seksual. Kurangnya informasi dan pengetahuan kalangan medis dan masyarakat tentang AIS sering mengakibatkan salah diagnosis dan salah manajemen.

Penelitian ini merupakan penelitian deskriptif retrospektif dan prospektif tentang gambaran klinik, hormon, sitogenetika, dan molekuler dari subyek dengan suspek AIS di laboratorium molekuler dan sitogenetika di Semarang dari April 2004 sampai April 2005. Data catatan rekam medik diperoleh dari Unit Molekuler dan Sitogenetika Fakultas Kedokteran Universitas Diponegoro dan Laboratorium Sitogenetika RS Telogorejo.

Pemeriksaan Quigley pada 46 kasus *partial* AIS (PAIS) menunjukkan 18 dengan stadium 3 (39,1%); 16 kasus stadium 2 (34,8%); 6 kasus stadium 4 (13%); 3 kasus stadium 6 (6,5%); 2 kasus stadium 1 (4,3%) dan 1 stadium 5 (2,2%). Duabelas individu dibesarkan sebagai perempuan, dan dua diantaranya dioperasi penyesuaian gender menjadi laki-laki. Terdapat dua keluarga besar dengan kecurigaan PAIS familial. Delapan kasus (16,33%) menunjukkan karakteristik hormon penderita PAIS. Empat dari sembilan subyek yang diperiksa menunjukkan mutasi *missense* R840H dan I603N.

Mendiagnosa PAIS dibutuhkan suatu pemeriksaan yang komplek, tidak hanya pemeriksaan fisik tetapi juga dapat dipertimbangkan pemeriksaan hormon, sitogenetik dan analisa molekuler. Mayoritas pasien datang dari latar belakang sosial ekonomi yang lemah, mahal biaya dan terbatasnya fasilitas pemeriksaan hormonal dan molekuler merupakan masalah utama dalam penegakkan diagnosis.

Kata kunci: Sindrom androgen insensitif, gambaran klinik, hormon, mutasi gen-AR.

INTRODUCTION

Sex differentiation and determination is a complex process. It follows a logical cascade, starts from the chromosomal sex determines the gonadal sex which is determines the phenotypic sex¹. In male sexual determination, Y chromosome especially SRY gene plays important role in changing the gonad into testis and the testis will produce hormones for determining male phenotypic sex.

One of the important hormones in male sexual differentiation is androgen. It is a steroid hormone which is synthesized from cholesterol. The two most important androgens are testosterone and dihydrotestosterone (DHT), which the latter is more potent². In order to work properly, androgen hormones need a receptor, called androgen receptor (AR). Androgen receptor encoded by the AR-gene, which is located in the long arm of the X-chromosome, Xq 11-12^{3,4}. Adequate androgen secretion as well as expression of a normal androgen receptor is important for normal male sexual differentiation and secondary sexual characteristic^{2,3}.

Androgen Insensitivity Syndrome (AIS) is an X-linked disorder of absent or defect virilization in 46, XY individuals due to the mutation of AR gene³. In this condition, although the androgen is produced sufficiently, but the peripheral masculinizing effect is blocked^{5,6}. The mutation can be caused either by inheritance or by spontaneous mutation. Most of the cases (70%), AR mutations are transmitted in an X-linked recessive manner through the carrier mothers, but the remaining mutations (30%) arises de novo⁷.

There are two types of AIS, complete and partial type^{8,9}. Patients with partial AIS (PAIS) cause a wide spectrum of phenotype, ranging from mildly virilized female external genitalia to mildly undervirilized male external genitalia¹⁰. Individuals with complete AIS (CAIS) will show a complete female external genitalia, including the breast development at puberty even a female psychosexual orientation^{8,11,12}.

Individuals with AIS will get larger psychological problems compare to the medical morbidity. This problem would be bigger if they should change their gender and need adaptation for the changing of sexual orientation. AIS and also the other sexual ambiguity usually ended by surgery. It is maybe the only way to solve their conflict by having their known gender. Reconstructive surgery such as vaginoplasty can be done for CAIS, but for PAIS, sometimes it is hard to determine the gender especially patients who come late. The delay of seeking medical treatment is caused by the lack of awareness from the patients, parents, and even the medical society.

Until now, the incidence of AIS in Indonesia is still unknown. A recent survey done in the Netherlands over a ten year period, from 1984-1993, based on reported cases of AIS indicated a minimal incidence of 1:99.000³. From the BBC health reports that in UK at least 1 from 24.600 babies are born with AIS.

There is a Sexual adjustment team in Dr. Kariadi university hospital and working group on ambiguous genitalia in Medical Faculty Diponegoro University. Most ambiguous genitalia cases from other hospitals in central Java are usually referred to this hospital. All members of this multidisciplinary gender team are working together to manage all ambiguous genitalia (intersexual) cases. All cases from each department (usually from Urology, Pediatric and Obstetric- Gynecology department) are always sent to the molecular and cytogenetic laboratories for chromosome and hormonal analysis before being discussed by the gender team. There are 2 cytogenetic laboratories in Semarang namely Molecular and Cytogenetic Laboratory of Medical Faculty

Diponegoro University and Cytogenetic Laboratory of Telogorejo hospital which is in linked each other.

The aim of this research is to show the frequency of AIS patients, as well as the hormonal profile, variant of phenotypes and also the mutation in Indonesian people. It can provide advantages not only for the medical society, but also as information for parents and communities. The research for this disorder in Indonesia is scanty consequently extended research for AIS should be continued to get more accurate data.

SUBJECT AND METHODS

Subjects

Subjects for this research are all ambiguous genitalia patients sent to two cytogenetic laboratories in Semarang. Target populations are all AIS patient clinically and hormonally diagnosed. This research was using a secondary data from medical records of AIS patients during period of April 2004- April 2005. Data was obtained from physical examination, hormonal assay, cytogenetic and mutation analysis.

Methods

This was a descriptive-observational retrospective and prospective study research. This research has been conducted in two places: Cytogenetics and Molecular Unit of Biotechnology Laboratory of Medical Faculty of Diponegoro University Semarang and Cytogenetics Laboratory of Telogorejo Hospital Semarang.

Patients diagnosed as AIS, then examined for further investigation. Physical examination includes Quigley stage, urethrogenital swelling, phallus length, localization meatus urethrae, presences of secondary sexual sign. Gender type, pedigree and photograph on genitalia were also recorded. Heparinized and EDTA peripheral blood was drawn for chromosome and DNA studies. Chromosome analysis was done using common G-banding technique. DNA was extracted from leucocytes of EDTA blood using salting out method ²¹. Subsequent DNA and plasma were sent to Erasmus Medical Centre for hormonal analysis and mutation analysis.

Data processing on physical measurement and result of cytogenetic analysis was analyzed with descriptive method SSPS 11.0 program. The result of data processing was reported in table and chart

RESULTS

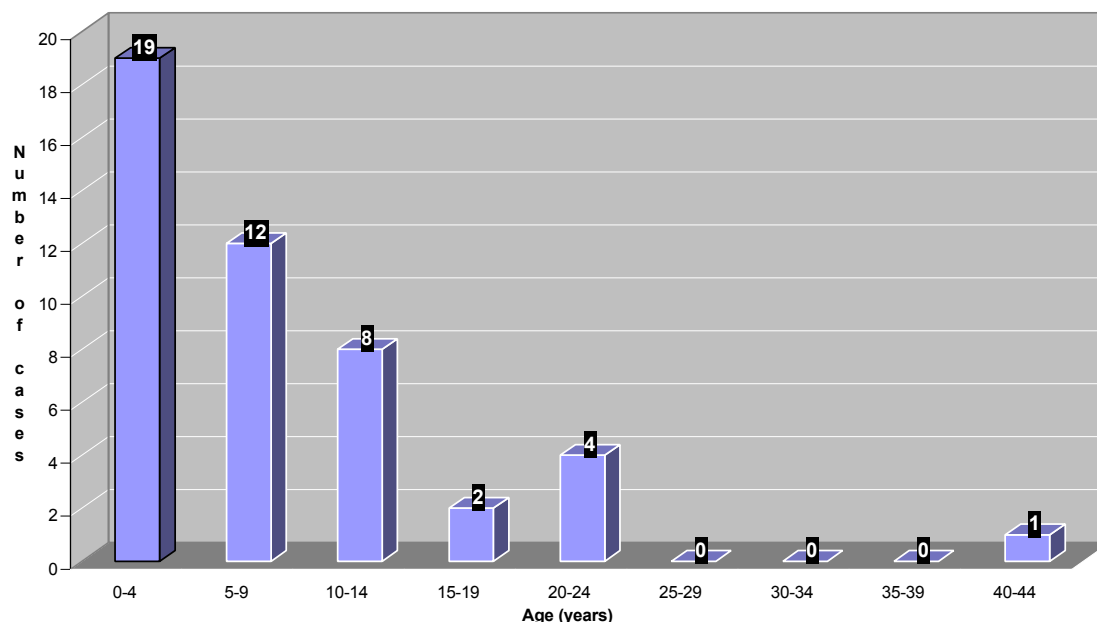


Figure-1. Distribution of age in patients with partial AIS in Semarang (N=46)

From all of 46 cases were Partial AIS (PAIS) with 46, XY. In figure-1, we distribute age of the patient into 9 classes. This was the age when the patient checked up for the first time. The youngest was in age 2 months, and the oldest was in 42 years old.

Quigley stage was used in classifying the external genitalia. Eighteen cases came with Quigley stage 3 (39,1%), 16 cases with Quigley stage 2 (34,8%). There were 6 cases (13%) with Quigley stage 4, 3 cases (6,5%) with Quigley stage 6, 2 cases (4,3%) with Quigley stage 1 and 1 case (2,2%) in stage 5 (see table-1)

At the time of diagnosis, the length of phallus of prepubertal subjects, all with male gender, ranged between 1,5 cm and 5,4 cm. Two prepubertal subjects with female gender had phallus length 2 cm and 3,7 cm. Among subjects examined during puberty, phallus measured ranged between 1,5 cm and 7,1 cm in subjects with female gender. Among the male gender at puberty, the phallus length ranged between 3 cm and 6,5 cm. In post

pubertal stage, phallus measured 2 cm and 5,9 cm in two subjects with female gender, while in male gender, phallus length was 3,4 cm and 4 cm.

Table-1 Profile of patients with PAIS

| No | Age (yr) | Gender | Hormonal Data | | | Mutation | Q.S | No | Age (yr) | Gender | Hormonal Data | | | Mutation | Q.S |
|----|----------|---------------|---------------|-----|-----|----------|-----|----|----------|--------|---------------|-----|-----|----------|-----|
| | | | LH | FSH | TSN | | | | | | LH | FSH | TSN | | |
| 1 | 9 | M | < | N | < | | 2 | 24 | 1 | M | < | < | < | | 4 |
| 2 | 15 | M | > | > | > | | 3 | 25 | 3 | M | < | < | < | | 4 |
| 3 | 12 | F | N | > | > | | 3 | 26 | 7 | F | N | > | > | | 3 |
| 4 | 17 | M, formerly F | N | > | < | | 2 | 27 | 1.5 | M | < | < | < | | 3 |
| 5 | 26 | M, formerly F | > | > | > | R840H | 3 | 28 | 14 | F | > | > | > | | 2 |
| 6 | 16 | M | > | N | > | R840H | 2 | 29 | 11 | M | < | N | < | | 3 |
| 7 | 21 | M | > | > | > | R840H | 2 | 30 | 14 | M | N | N | > | | 2 |
| 8 | 10 | M | N | > | N | | 1 | 31 | 5 | M | < | N | < | | 4 |
| 9 | 13 | F | > | > | < | | 6 | 32 | 10 | M | < | N | > | | 3 |
| 10 | 2 | M | < | < | < | (-) | 2 | 33 | 14 | M | N | > | > | | 2 |
| 11 | 2.5 | M | < | < | < | | 3 | 34 | 10 | M | < | < | < | | 3 |
| 12 | 12 | M | N | N | < | I603N | 5 | 35 | 6m | M | < | N | < | | 3 |
| 13 | 14 | F | > | > | < | (-) | 3 | 36 | 3 | M | < | < | < | | 2 |
| 14 | 8 | M | N | N | > | (-) | 4 | 37 | 23 | F | > | N | N | | 3 |
| 15 | 7 | M | < | < | < | | 3 | 38 | 5.5 | M | < | < | < | | 3 |
| 16 | 23 | F | > | > | < | (-) | 6 | 39 | 42 | M | > | > | N | | 3 |
| 17 | 2 | F | N | < | > | (-) | 4 | 40 | 4 | F | < | < | < | | 4 |
| 18 | 4m | M | < | N | < | | 2 | 41 | 1.5 | M | < | N | > | | 3 |
| 19 | 10 | M | < | N | < | | 2 | 42 | 12 | M | < | N | < | | 2 |
| 20 | 4 | M | < | < | < | | 2 | 43 | 2.5 | M | < | N | > | | 3 |
| 21 | 2 | M | < | N | < | | 1 | 44 | 2.5 | M | < | N | > | | 3 |
| 22 | 7 | M | < | < | < | | 2 | 45 | 4.5 | M | < | < | N | | 2 |
| 23 | 2 | M | < | < | < | | 2 | 46 | 22 | F | > | > | < | | 6 |

Information :
LH : Liutenizing Hormone
FSH : Follicle Stimulating Hormone
TSN : Testosterone
Q.S. : Quigley Stage
< : Lower than normal
> : Higher than normal
N : In normal range

Ten individuals were raised as female, and 34 individuals were raised as male, while two individuals had been operated to adjust the gender from female into male (see table-1). From ten female individuals, there were 5 of them which have introitus vaginae. And from those individuals with introitus vaginae, there were 1 individuals which has uterus, but without cervix uteri.

Breast development was found in four post pubertal subjects. One of them had already removed because of gender adjustment. Pubic hair development was found in 13 subjects.

All of those patients with partial AIS had been taken their bloodsample to analyze hormones. The interpretation of the hormonal data can be seen in table-1. The interpretation includes of 3 hormones, LH, FSH, and testosterone. From the hormonal data, the hallmark of AIS was the increase of LH hormone, and also the testosterone can be slightly high or normal³. Thus, from 46 cases, 7 of them were diagnosed as AIS from the hormonal status.

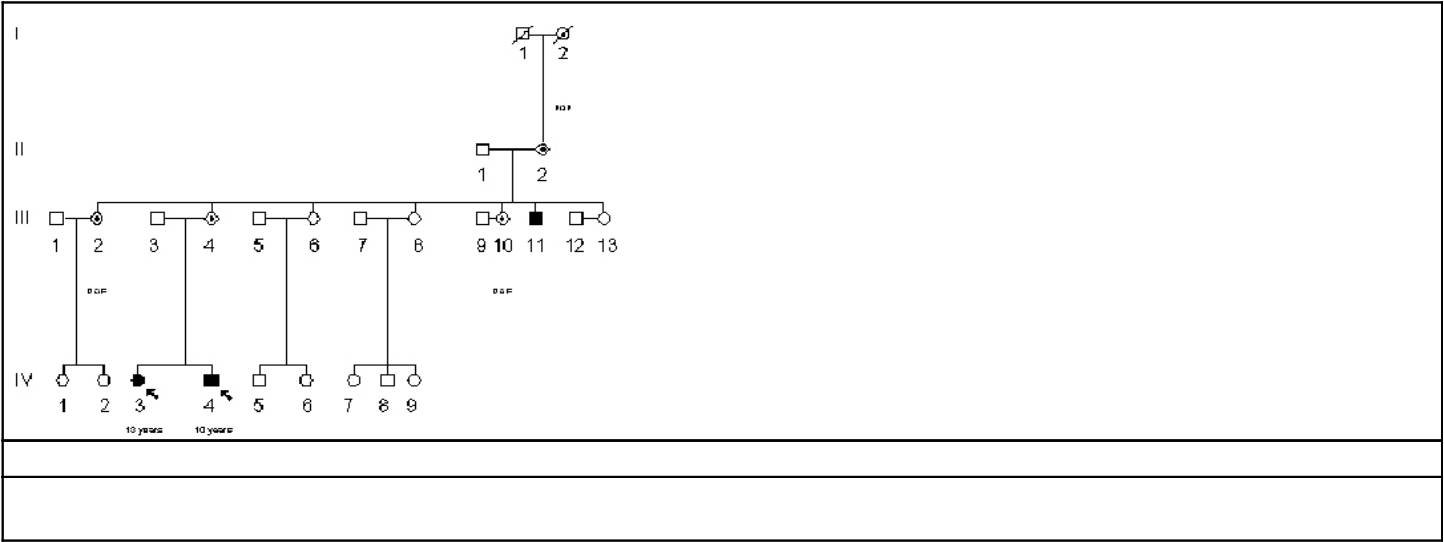
Beside the hormonal data, the DNA was analyzed in Rotterdam resulting 4 cases with missense

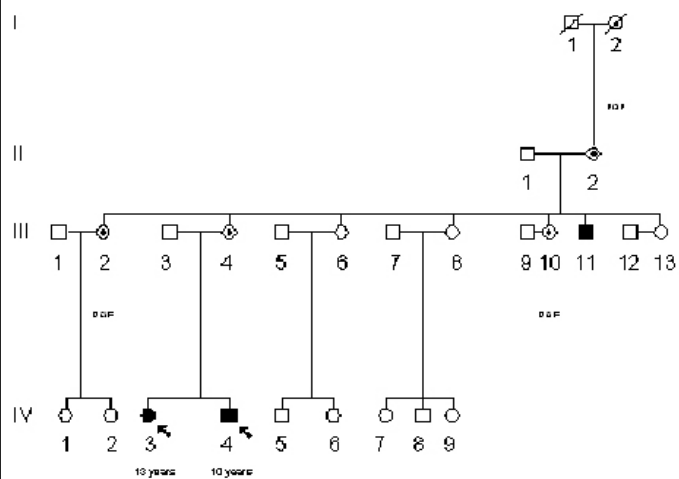
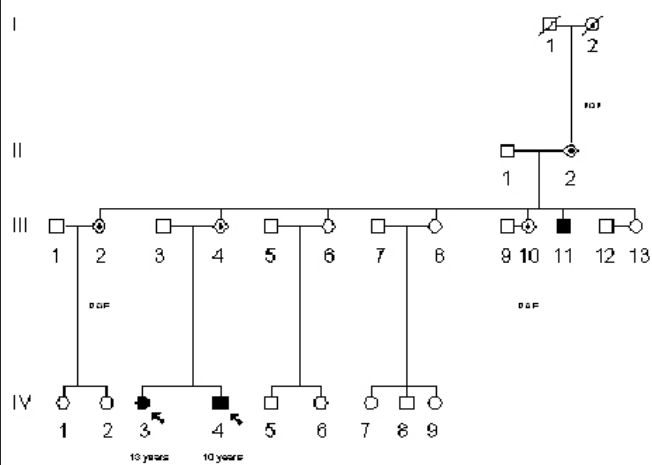
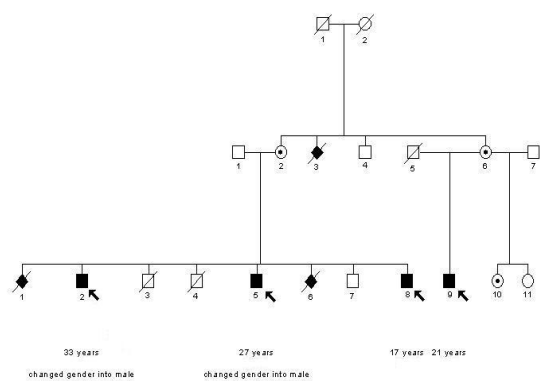
mutations in the AR-gene (see table-1). Subject no. 5, 6, and 7 were one family (see also family-B) and shown that mutations of R840H occurs. It means that there was a substitution at codon 840 that resulted in substitution of arginine (R) into Histidine (H). In subject 15, DNA analysis was shown I603N i.e. substitution of Isoleusin (I) into asparagine (N) on codon 603.

The other DNA analysis had been done also in subject number 10, 13, 14, 16, 17. Mutation analysis did not result in the identification of a well known mutation of the androgen receptor gene (see table-1). The result did not confirm the diagnosis complete/partial androgen insensitivity syndrome, but the result did not exclude this diagnose either, cause it could be a mutation in somewhere else in the AR gene.

There are two large families that suggested the X-linked of AIS. Not all of the family members could be studied because they live in different places. The pedigree from both families can be seen in figure-2 below.

There were three subjects with partial AIS in family A. Premature ovarian failure (POF) was running in this family. Subject IV-3 (no 9), 13 years old, was raised as female with Quigley stage 6, with normal labia majora and minora and blind end vagina. At the diagnosis was made, subject IV-3 was already operated for inguinal testes and clitoris adjusment. Subject IV-4 (no 8), 10 years old, Quigley stage 1, had scrotum with testes right and left, phallus measured at 5.4 cm. Their uncle, subject III-11, 33 years old, had a feminine habitus and already married for 6 years, but without a child. Subject III-11 was not included in the hormonal assay above, but the DNA analysis for AR-gene was made and there were no mutation found on the well known region, but this result could not exclude the diagnosis probably there is a mutation somewhere along the AR-gene.





There were four subjects from family B. Hormonal data of Subject III-2 (diagnosed as PAIS) was not available because of the rejection of the second blood collection. Subject III-5 (see figure-3), age 27, also had gyanecomastia, feminine habitus with introitus vaginae, but without uterus, phallus length measured at 2,6 cm, and also had pubic and axillair hair development. Subject III-2 and III- 5 changed their gender after they are getting adult because of the androgen exposure made their body posture changed to male. The operation was made not only for the hypospadia but also to change their gender to male. Subject III-8 (no 6) from family B, 17 years old, diagnosed with Quigley stage II, had scrotum, phallus length was 3,7 cm and had hypospadia scrotalis. Subject III-9 (no 7), 21 years old, with Quigley stage II, had scrotum bifid, 3,4 cm of phallus length, and had hypospadia scrotalis. Both subjects showed hair development in pubic and axillair. Mutation R840H found in subject III-5, III-8 and III-9.



DISCUSSION

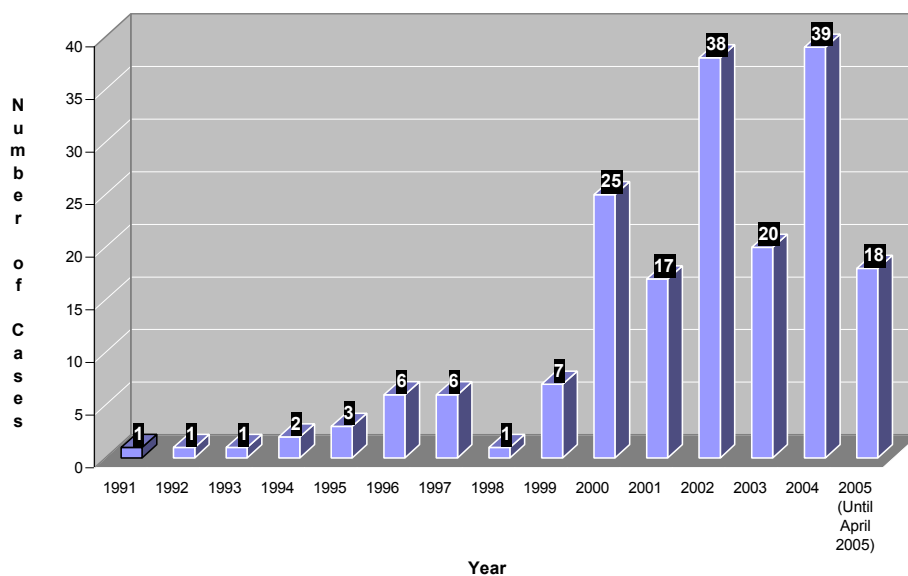


Figure-4. Total Sexual Ambiguous and Hypospadias Cases in Cytogenetic Laboratories in Semarang per Annum (Oct 1991-April 2005, N=185)

Until April 2005, 185 cases of sexual ambiguity and hypospadias have recorded in Cytogenetic Laboratories in Semarang. There were 93 cases (50,27%) from the Telogorejo Hospital and 92 cases (49,73%) from the dr. Kariadi Hospital. There was a tendency to increase the case in each year (see figure-4) although it can not be denied that there are still many more cases that beyond our reach. This can be caused by better information to medical and public communities via symposia, seminars and workshops that make medical society more aware¹⁵. This awareness can prevent the late diagnosis or even misdiagnosis.

It is hard to detect and diagnose PAIS, since the phenotype are varies among the cases. Cases whose were raised as female, their external genitalia showed virilization, while the internal genitalia showed regression because of the presence of AMH (Anti Mullerian Hormon) or MIS (Mullerian Inhibiting Substance). Hypospadias and scrotum bifidum were found in almost all male cases. It proves that male sexual differentiation was not completed.

There are twelve individuals (26,09%) which are raised as female. Therefore, gender assignment should be preceded by the cytogenetic examination. Establishing the diagnosis of PAIS is very important for gender assignment to an infant of ambiguous genitalia¹⁶. Gender adjustment was done in very young age in developed countries, but in this research the gender adjustment was done after they are getting puberty.

The pubic and axillair hair development were varied. It can be understood because androgen acts on hair follicle, especially in pubic, axillair, chest and face. Otherwise in complete AIS, where there are no androgen actions in the body, the absence or minimal pubic and axillair hair is a stronger sign of CAIS⁴. However the connection between the AR activity and the pubic hair development is still unclear, it is suggest that the pubic hair may develop independently from AR activity¹⁷. The descending of the testis, in almost all cases completed. Even still in some cases, the testis was refractile or can be moveable into inguinal. This indicates an important, but not absolute, role of androgen in testicular descent.

This study also reveals two types of mutation. In R840H mutation, the hormonal profile of the subject showed the characteristic of PAIS. From the literature, R840 is the codon with the greatest number of different amino acid substitutions in AIS. There are three other types of mutation in this codon, R840G, R840S, R840C¹⁸. Each of these mutations was associated with a very diverse spectrum of phenotypes¹⁹.

Subject no 12 with I603N mutation, the LH level was normal and testosterone level was low, but phenotypically as a male, indicated Quigley stage 5, had the introitus vagina and ended blindly. On the other hand, three subjects with R840H mutation showed Quigley stage 2 and 3. Subject with Quigley stage 3 also had introitus vaginae and feminine habits and already been operated to adjust the gender from female into male. Two remaining subjects showed phallus length 3,4 and 3,7 cm, scrotal hypospadias, and also pubic and axillair hair development. This result shows that PAIS displays a large phenotypic variation, and molecular analysis does not always explain the variance of the phenotype^{3,20}. More research in AIS probably could reveal the pattern and the connections between genotype and phenotype.

All of the cases in this study were PAIS. An interesting case were subject 16 who raised as female, on physical examination showed complete female external genitalia (Quigley 6), but also showed Adam apple and low voice. Hormonal study showed LH and FSH increased but the testosterone level was decreased. Molecular analysis did not show any common mutation. This subject needs further discussion since the physical examination indicated the sign of AIS.

Along with hormonal and clinical findings for the other subjects still need molecular analysis in order to make accurate diagnosis. For those patients with no common mutation found does not exclude the diagnosis, because the mutation could be in the other region that should be explored.

Physical examination, hormonal, cytogenetic and molecular studies in patients with PAIS should be undertaken to established accurate diagnosis¹⁵. Unfortunately, in Indonesia, molecular diagnostic test is limited due to the lack of facilities¹⁵ and most of the patients come from low economic level, therefore sometimes AIS is diagnosed only based on cytogenetic and physical examination. Cooperation with other center is very important in order to diagnose AIS more accurately. Over time, AIS patients either complete or partial have more risk of malignant degeneration of the testis^{3,10}. Gonad surgery and long life hormonal therapy should be accompanied by

genetic counseling, psychological approach and also support from family for treatment of patients with PAIS.

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